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Reexamination on the Taxonomic Position of Two Intraspecific Taxa in Japanese *Eothenomys*: Evidence from Crossbreeding Experiments (Mammalia: Rodentia)

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ABSTRACT—As a part of the study in which the taxonomic validity of *E. kageus* is inclusively reexamined, crossbreeding experiments were made between *Eothenomys smithii* with six mammae and *E. kageus* with four mammae, which have been separated by the difference mainly in number of the mammae and in shape of the baculum. Consequently, these two species readily interbred in crosses of both *E. smithii* ♀ × *E. kageus* ♂ and *E. kageus* ♀ × *E. smithii* ♂. F_1 hybrids obtained from the two crosses possessed normal breeding ability. Moreover, F_5 hybrids were produced from the line of the former cross. Some daughters were different from their mothers in number of the mammae, and some litters included both females with four mammae and females with six mammae within a litter. These facts suggest that both species may share an intercommunicating gene pool with each other, and mean that the difference in number of the mammae is an intraspecific variation or a polymorphism. It has been also said that the shape of the bacula is a poor taxonomic character. Therefore, taking the reproductive compatibility between them and the unreliability of the taxonomic characters into account, together with the almost entire identity in their karyotypes previously reported by us, it is concluded that *E. kageus* is synonymous with *E. smithii*.

INTRODUCTION

Taxonomy must be grounded on integrated conclusions which were clarified by investigations from various aspects. Accordingly, when two related forms, which had been separated only by the morphological characters, have given rise to a taxonomic dispute, more extensive studies are desired. In particular, a crossbreeding experiment occupies a great important position in this field and is a useful method of assessing the relationship between such allied forms.

The Smith's red-backed vole with six mammae (*Eothenomys smithii*) and "Kage" red-backed vole with four mammae (*E. kageus*) are said to occur in the western and central parts of Japan, respectively [1]. However, a dispute on the taxonomic position of these two "species" has still remained unsolved. The dispute began with the separation of *E. kageus* from *E. smithii* by the difference mainly in number of the mammae and in shape of the baculum. Afterwards, many studies have been made on this problem, but restricted to morphological [2–7] and karyological (a conventional staining method) [8, 9] aspects. In this connection, as a part of the broader study in which the taxonomic validity of *E. kageus* is inclusively reexamined, comparisons of the growth and development patterns and the karyotypes (G- and C-band patterns) between *E. smithii* and *E. kageus* revealed that the characteristics of these patterns in *E. kageus* were basically identical with those in *E. smithii* [10, 11]. Then, in order to gain new information on this problem, crossbreeding experiments between them were carried out, although the sample size was small. The aim of the present study is to examine the reproductive compatibility between *E. smithii* and *E. kageus* and the reliability of the number of the mammae as a diagnostic character, and to discuss the taxonomic position of both

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species.

MATERIALS AND METHODS

E. smithii (2 males, 6 females) and *E. kageus* (3 mles, 2 females), used as parental generations, were obtained from laboratory colonies which were derived from wild voles live-trapped in Kyushu (Fukuoka Prefecture) and central Honshu (Nagano and Yamanashi Prefectures), respectively. All hybrid generations were originated from four pairs of *E. smithii* ♀ × *E. kageus* ♂ and two pairs of *E. kageus* ♀ × *E. smithii* ♂ (see Table 1).

All animals were housed in stainless steel cages (43×25×23 cm), and given *ad libitum* a commercial diet (NMF or CMF, Oriental Yeast Co., Ltd., Tokyo) and water, and sometimes fresh cabbages. The experimental colonies were maintained at temperatures of 20±1°C on photoperiods of 12 hr light: 12 hr dark. Humidity was not controlled throughout the experimental period. Voles produced litters throughout the year under such rearing conditions. Gravid females were inspected daily for deliveries so that their litters were discovered within 24 hr *post partum*. The day when neonates were found by checking was designated the day of parturition and day 0 of the newborn young. The number of young on the day when they were found was regarded as the litter size. Since the mammae of female young became detectable as about day 7 in both *E. smithii* and *E. kageus* [12, 10], the number of the mammae of female hybrids was determined at this time.

RESULTS

The results of the breeding experiments are given in Table 1. Eleven types of crosses, consisting of eight in the line of *E. smithii* ♀ × *E. kageus* ♂ and three in the line of *E. kageus* ♀ × *E. smithii* ♂, were attempted in this study. Litters were produced from all types of crosses. Mean litter sizes showed 2.5–3.0 in most of the crosses, although the smaple size was very small in some cases. The maximum and minimum mean litter sizes were 4.8 and 1.9, respectively.

Regarding the number of the mammae, there were two kinds of crosses; i.e. one was the cross in which all females of the progeny had six mammae, and the other was the cross in which females of the progeny possessed four or six mammae. Out of the eleven types of crosses, eight types of crosses leading to the former case bore 71 litters *en bloc*, whereas the remaining three types of crosses resulting in the latter case gave birth to 29 litters as a whole. Out of the above 29 litters, five litters contained both females having four mammae and females having six mammae (6 males and 12 females in total; out of 12 females, five had four mammae and six had six mammae, and one was undetermined); the remaining 24 litters involved only females with six mammae. However, no cross existed in which all female hybrids had four mammae. The maximum longevity was 1,354 days in F_1 hybrids between *E. smithii* ♀ and *E. kageus* ♂.

DISCUSSION

1) *Reproductive compatibility between* E. smithii *and* E. kageus.

Isolating mechanisms which are necessary for maintenance of speices integrity are classified into two categories, premating and postmating mechanisms; the former category contains seasonal and habitat, ethological and mechanical isolation, while the latter category includes gametic and zygote mortality, hybrid inviability and hybrid sterility [13].

In connection with attempts to obtain interspecific hybrids, crossbreeding (or artificial insemination) experiments between different species have been carried out in some orders of Mammalia, including Rodentia. However, there have been few hybrids with fertility in such cases because of operation of the above postmating mechanisms. As examples of hybrids which die during embryogenesis, the following combinations have been well known: goats (*Capra hircus*)× sheep (*Ovis aries*) [14], ferrets (*Mustela furo*)× minks (*Mustela vison*) [15], rabbits (*Oryctolagus cuniculus*)×hares (*Lepus americanus*) [15] and black rats (*Rattus rattus*)×brown rats (*Rattus norvegicus*) [16,17]. On the othr hand, in crosses of horses (*Equus caballus*)×donkeys (*Equus asinus*) [18], horses (*E. caballus*)×zebras (*Equus grevyi*)

TABLE 1. Breeding results

	Types of crosses	No. of pairs	No. of litters	No. of young ♂	No. of young ♀	No. of young Ud	Mean litter size	Range	No. of mammae of ♀ young
I	*E. smithii* (♀)×*E. kageus* (♂)	4	17	27	20	2	2.9	1–5	6
	(*smithii*×*kageus*)F_1×(*smithii*×*kageus*)F_1	2	16	24	25	4	3.3	1–5	6
	(*smithii*×*kageus*)F_2×(*smithii*×*kageus*)F_2	5	17	24	19		2.5	2–4	6
	(*smithii*×*kageus*)F_3×(*smithii*×*kageus*)F_3	3	12	14	15	1	2.5	1–5	4 or 6*
	(*smithii*×*kageus*)F_4×(*smithii*×*kageus*)F_4	1	2	2	3		2.5	2–3	6
	E. smithii (♀)×(*smithii*×*kageus*)F_1 (♂)	2	7	6	7		1.9	1–3	6
	(*smithii*×*kageus*)F_3 (♀)×(*smithii*×*kageus*)F_2 (♂)	1	1	1	2		3.0	3	4 or 6†
	(*smithii*×*kageus*)F_4 (♀)×(*smithii*×*kageus*)F_3 (♂)	1	2	3	2		2.5	2–3	6
II	*E. kageus* (♀)×*E. smithii* (♂)	2	8	13	15	2	3.8	2–6	6
	(*kageus*×*smithii*)F_1×(*kageus*×*smithii*)F_1	3	16	40	33	3	4.8	3–6	4 or 6‡
	(*kageus*×*smithii*)F_2×(*kageus*×*smithii*)F_2	1	2	3	3		3.0	3	6

Ud, undetermined.

* Two and eight females have 4 and 6 mammae, respectively, and five have mammae of undetermined number.

† One and the other female have 4 and 6 mammae, respectively.

‡ Four and 23 females have 4 and 6 mammae, respectively, and six have mammae of undetermined number.

[19], Syrian hamsters (*Mesocricetus newtoni*)× golden hamsters (*Mesocricetus auratus*) [20] and Shaw's jirds (*Meriones shawi*)×Libyan jirds (*Meriones libycus*) [21], their hybrids survive to adulthood, but males and/or females are sterile.

As to the present experiments, in crosses of both *E. smithii*♀×*E. kageus*♂ and *E. kageus*♀×*E. smithii*♂, these two species readily interbred. Both sexes of F_1 hybrids obtained from the above two crosses seem to possess, at least under laboratory conditions, normal breeding ability. Further, F_5 hybrids were produced from the line of the former cross. Therefore, these facts demonstrate that almost no barrier caused by postmating isolating mechanisms exists between both species, so far as the crossbreeding experiments are concerned.

In our study mean litter sizes in the crosses were 1.9–4.8, being smaller than the mean litter size (4.5) of *E. smithii* [22], except for the value 4.8. *E. smithii* has wide individual variations in litter size, and the lowest and highest prolificacies were 2.8 and 6.8 young per litter, respectively [22]. Accordingly, when the litter size is discussed, it is necessary to get a larger sample size just in such crossbreeding experiments. Although there is still much to be investigated, very important is the fact that progeny between *E. smithii* and *E. kageus* were fertile. This fact suggests the probability that both species may share an intercommunicating gene pool with each other, but detailed analyses at the gene level are indispensable with respect to this.

It must be noted that fertility or sterility of hybrids is not the sole criterion of species [13, 23]. For example, coyotes (*Canis latrans*)×dogs (*Canis familiaris*) hybrids are fertile [24]: premating isolating mechanisms seem to play a significant role in such cases [13]. There has been no direct evidence showing the presence or absence of barriers due to premating mechanisms between *E. smithii* and *E. kageus* under natural conditions. However, judging from our success in interbreeding between both species in the laboratory, and from existence of females with four and six mammae under both laboratory and field (as mentioned below) conditions, there is a high possibility that both forms are crossbred under natural conditions.

2) *Taxonomic validity of* E. kageus.

When discussing the taxonomic status of *E. smithii* and *E. kageus*, it is of importance to examine the reliability of the taxonomic characters

(the number of the mammae and the shape of the baculum) which were described by Imaizumi [1].

Imaizumi [1] has stated that the difference in the mammary formula of these two species seems to be fairly important as one of the main diagnostic characters. However, our crossbreeding experiments revealed that some daughters were different from their mothers in number of the mammae, and that some litters included both females with four mammae and females with six ones within a litter. These facts apparently indicate that the number of the mammae is not a constant character at the individual level. Furthermore, in Mt. Yatsugatake (Nagano Pref.), Mt. Kamegamori (Ehime Pref.), Mt. Tsurugi (Tokushima Pref.) and Mt. Hakusan (Ishikawa Pref.), both females with four mammae and females with six mammae are captured at the same locality [3–5, 25]. The difference in number of the mammae has been considered to be individual variation [3, 4]. In this context, it is worthy of note that a pair of voles from Nagano Prefecture (the female with four mammae) produced a litter including a female with four mammae and a female with six mammae in our laboratory [unpublished]; this fact agrees with the results of the above field studies.

In murid mammals, the swamp rat (*Rattus lutreolus*) and the bush rat (*Rattus fuscipes*) inhabiting Australia are known as species in which the numbers of the mammae are different between geographically isolated populations and between subspecies, respectively [26]. In the former species, mainland females have five pairs of teats, while females from Tasmania have only four pairs. *Rattus fuscipes* is divided into four subspecies (*R. f. fuscipes, R. f. greyi, R. f. assimilis* and *R. f. coracinus*); only *R. f. coracinus* females have four pairs of teats, whereas females of the other three subspecies have five pairs. Thus, the difference in number of the mammae is an intraspecific variation or a polymorphism, being unreliable as a diagnostic character by which *E. kageus* was separated from *E. smithii.* With respect to this problem, Kaneko [7] also has drawn the same conclusion from an investigation on the number of the mammae in pregnant or postpartum wild females.

Regarding the baculum, Imaizumi [1] has mentioned that there are differences in general outline of the posterior border of its body (semicircular in *E. smithii,* while concave in *E. kageus*) and in shape of the lateral prongs (curved as the letter "c" in *E. smithii,* while double curved as the letter "s" in *E. kageus*). However, Jameson [2] has stated that the difference in the bacula of *E. kageus* and *E. smithii* may be due to individual variation. On the basis of a detailed analysis of the bacula in 71 wild males, Kaneko [7] also has concluded that the above differences in the bacula cannot be employed as a diagnostic character between these two species in question.

Furthermore, the following facts have been reported: in an analysis of the skull, the relative growth coefficient shows no difference among five localities including Kyushu and central Honshu [6]; geographical clines are recognized in the hind foot length, the tail length and the parietal width [4, 6]; the growth and development patterns of both species are basically identical with each other [10]; except for a slight variation in size of the short arm of the Y chromosome, no detectable difference is found in the karyotype (G- and C-band patterns) between both species [11]. All these facts may indirectly adduce negative evidence for the taxonomic validity of *E. kageus.*

From the above consideration, it can be said that *E. kageus* may not be reproductively isolated from *E. smithii,* even under natural condition, and that the taxonomic characters (the number of the mammae and the shape of the bacula) pointed out by Imaizumi [1] are not regarded as diagnostic. Our conclusion reached, therefore, is that *E. kageus* is synonymous with *E. smithii.*

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